SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF COLLOIDAL SILVER NANOPARTICLES ENCAPSULATED IN LOW GENERATION POLY(AMIDO)AMINE DENDRIMERS

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For my beloved parents,
Hamidon Bin Sapee and Rasidah Abd Lah
My beloved sisters,
Nurul Faziha and Nurul Hamimzah
My supportive brother in laws,
Mohd Khairi and Muhammad Safuan
My Fiancee,
Muhammad Hafidz Bin Hamidi

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ABSTRACT

Poly(amidoamine) (PAMAM) dendrimers of low generations 0.5, 1.0, 1.5, and 2.0 were synthesized in methanol via the divergent synthesis method, which includes alternating reiteration of Michael addition followed by ester amidation starting from ethylenediamine and methyl acrylate. PAMAM dendrimer encapsulated with silver nanoparticles were prepared by *in situ* reduction of $AgNO_3$ solution with $NaBH_4$ in the presence of PAMAM dendrimers. This procedure resulted in stable yellowish colloidal solutions which turn into dark brown colloid and precipitated as deep black solid silver(I) oxide, Ag_2O upon exposure to light. Verification of the PAMAM structures was done using fourier transform infrared (FT-IR) spectroscopy, and ^1H nuclear magnetic resonance (NMR) spectroscopy. The results of ^1H NMR spectra of synthesized PAMAM indicated that some structural defects were present in the form of missing –NH groups presumably due to incomplete amidation or intramolecular cyclization of terminal amino groups. Characterization of PAMAM-silver nanocomposites by ultra-violet visible (UV-Vis) spectroscopy showed that the surface plasmon resonance (SPR) of colloidal silver shifted from approximately 414 nm to higher wavelengths suggesting specific interaction between PAMAM and silver nanoparticles. This is supported by the fluorescence spectra of generation 2.0 PAMAM-silver nanocomposite which exhibited quenching of the emission peaks of fluorophore at 510 nm after silver encapsulation. The particle sizes of colloidal silver at concentrations of 200 and 800 ppm were found between 5 and 20 nm as determined from transmission electron microscopy (TEM). In this study, it was demonstrated that low generation of PAMAM dendrimers able to act as template to pre-organize silver ions following *in situ* reduction with sodium borohydride ($NaBH_4$). PAMAM dendrimer of generation 2.0 was screened for its potential antibacterial activity against two bacteria, viz, *Escherichia coli* ATCC 11229 (Gram negative) and *Staphylococcus aureus* ATCC 6538 (Gram positive) using disk diffusion technique (DDT) and minimum inhibition concentration (MIC) method. Study on the effect of ratios of silver concentration to PAMAM generation 2.0 indicated that the antibacterial activity against both Gram negative and Gram positive bacteria was lower than for PAMAM-silver nanocomposite. The findings also indicate that the presence of silver in the dendrimer has further enhanced the antibacterial activity against Gram negative bacteria which was also dependent on the concentration of silver solutions. The higher antibacterial activity of PAMAM-silver nanocomposite could be due to strong interaction between negatively charged bacterial cell wall and the cationic PAMAM dendrimer, which possibly decrease the distance between silver nanoparticles and the bacteria. This interaction then enables the silver nanoparticles easily attached to the bacterial cell surface and even penetrate the cell walls, killing the bacteria resulting in the antimicrobial activity.

ABSTRAK

Dendrimer poli(amidoamina) (PAMAM) bergenerasi rendah 0.5, 1.0, 1.5, dan 2.0 telah disintesis dalam metanol melalui kaedah sintesis divergen yang meliputi pengulangan berselang seli penambahan Michael diikuti pengamidaan ester bermula daripada etilenadiamina dan metil akrilat. Dendrimer PAMAM terkapsul nanopartikel argentum telah disediakan secara penurunan *in situ* larutan akueus $AgNO_3$ menggunakan $NaBH_4$ dengan kehadiran dendrimer PAMAM. Prosedur tersebut menghasilkan larutan koloid kekuningan yang stabil membentuk koloid coklat tua dan mendakan hitam argentum(I) oksida, Ag_2O terbentuk selepas pendedahan kepada cahaya. Verifikasi struktur PAMAM dendrimer telah dilakukan menggunakan spektroskopi inframerah transfromasi fourier (FT-IR) dan spektroskopi resonans magnet nukleus (NMR) spektrum ^1H. Keputusan spektrum ^1H NMR untuk PAMAM yang disintesis menunjukkan beberapa kecacatan struktur wujud dalam bentuk kehilangan kumpulan -NH mungkin akibat disebabkan pengamidan yang tidak lengkap atau pembentukan gelang intramolekul kumpulan amino hujung. Pencirian nanokomposit PAMAM-argentum menggunakan spektroskopi ultralembayung-nampak menunjukkan resonans dapat dijelaskan plasmon permukaan (SPR) bagi koloid argentum beranjak dari sekitar 414 nm ke nombor gelombang yang lebih tinggi mencadangkan interaksi di antara PAMAM dan nanopartikel argentum. Ini disokong oleh spektrum pendafluor nanokomposit PAMAM generasi 2.0-argentum yang menunjukkan pelindap kejutan puncak pancaran fluorofor selepas pengkapsulan argentum. Saiz zarah argentum koloid pada kepekatan 200 dan 800 ppm didapati antara 5 hingga 20 nm seperti yang ditentukan oleh mikroskopi penghantaran elektron (TEM). Dalam kajian ini, telah ditunjukkan bahawa dendrimer bergenerasi rendah mampu bertindak sebagai templat untuk penyebaran ion argentum selepas penurunan *in situ* menggunakan natrium borohidrida ($NaBH_4$). PAMAM generasi 2.0 telah diuji potensi aktiviti antibakteria terhadap dua bakteria, *Escherichia coli* ATCC 11229 (Gram negatif) dan *Staphylococcus aureus* ATCC 6538 (Gram positif) menggunakan teknik resapan cakera (DDT) dan kepekatan perencatan minimum (MIC). Kajian tetap terhadap kesan nisbah kepekatan argentum kepada PAMAM generasi 2.0 menunjukkan aktiviti antibakteria terhadap kedua bakteria Gram negatif dan Gram positif adalah rendah berbanding nanokomposit PAMAM-argentum. Keputusan kajian juga menunjukkan kehadiran argentum meningkatkan lagi aktiviti antibakteria terhadap bakteria Gram negatif yang turut bergantung kepada kepekatan larutan argentum. Aktiviti antibakteria yang lebih tinggi bagi nanokomposit PAMAM-argentummungkin disebabkan oleh interaksi yang kuat antara dinding bakteria yang bercas negatif dan kation dendrimer PAMAM, yang mungkin mendekatkan jarak antara nanopartikel argentum dan bakteria. Interaksi membolehkan nanopartikel argentum melekat pada permukaan bakteria dan meresap masuk ke dalam dinding sel dan seterusnya membunuh bakteria tersebut dan menghasilkan aktiviti antibakteria.